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Gustatory responses in primates to the sweetener aspartame and their phylogenetic implications

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Abstract. Two-bottle preference tests have been applied to 70 (sub-) species of the order of Primates and, for comparison, to the tree shrew (*Tupaia belangeri*) to determine their responses to aspartame (APM), the first known sweet-tasting dipeptide which has to man a sweetness potency of about 200 times that of sucrose. It was found that only the Cercopithecidae, the Hylobatidae and the Pongidae respond like man to this dipeptide and prefer it to water. The other primates tested to date, show no response to this sweetener. From a phylogenetic point of view, we note that APM shows species specificity similar to thaumatin. Thus, a clear dichotomy exists within the order of Primates with respect to both thaumatin and APM. The results here illustrate once more the gustatory diversity among primates and are a compelling argument for the existence of different sweet taste receptors or recognition sites in primate species.

Introduction

In December 1965, James Schlatter of Searle Pharmaceutical Company discovered the sweet taste of N-a-L-aspartyl-L-phenylalanine methyl ester (m.w. 294 dalton). This finding opened up a new area of research in synthetic sweeteners (Mazur and Ripper, 1979). This first known sweet-tasting dipeptide was generically named 'aspartame' (APM) and has to man a sweetness potency of about 200 times that of sucrose. Since the discovery of APM, a great number of reports have been made on structurally related substances with widely ranging sweetness potencies. In addition to research at Searle (Mazur *et al.*, 1970, 1973), several other industrial groups have been active in the search for new sweeteners. Of particular note are the efforts at Unilever Research (Brussel *et al.*, 1975; van der Heijden *et al.*, 1978, 1979; and van der Wel *et al.*, 1987), Dynapol Company (DuBois *et al.*, 1977, 1981a, 1981b, 1984), Ajinomoto Company (Arioshi *et al.*, 1974; Arioshi, 1976), Pfizer Pharmaceutical Company (Glowaky *et al.*, 1991) and the Tate & Lyle Company (Jenner *et al.*, 1991). In all these publications, the structure/taste relationships reported are based on the activity in man.

The first report on the responsiveness of sweeteners other than man was that of Sato and co-workers (1977), in Japanese monkeys (*Macaca fuscata*). They found, by both behavioural and electrophysiological methods, that APM is about 10 times more effective in eliciting a response than sucrose and that the animals prefer this compound against water.

More extensive studies were reported a short time later by Hellekant and co-workers (1981) as are summarized in Table I. They studied the activity of six sweeteners, one of which was APM, in five different primate species. They found that in all five species

Table I. Comparison between the percentage consumed in a two-bottle preference test between aspartame and tap water and the amplitude of the summated electrophysiological responses elicited by aspartame. The number of observations is indicated by *n* (from Hellekant *et al.*, 1981)

Species	Aspartame - responses	
	% consumed	Ampl sum resp mV
Saguinus midas niger	46 ± 19 (n = 4)	30
Callithrix jacchus jacchus	40 ± 22 (n = 8)	—
Galago senegalensis	62 ± 20 (n = 4)	50
Nycticebus coucang	39 ± 21 (n = 4)	25
Tupaia belangeri	47 ± 14 (n = 8)	10

APM was neither rejected nor preferred relative to tap water and in four species caused no change in the chorda tympani nerve activity.

Thus, a contrast exists in the responsiveness of these different primate species to APM. This species specificity to the sweetener APM further defines the differences between mammalian species (Glaser *et al.*, 1978) and further illustrates the phylogenetic relationships within the order of Primates. Thus, APM responsiveness should be considered a significant adjunct for establishing primate phylogeny.

Materials and methods

A two-bottle preference (TBP) test was employed to judge preference to APM (+) or otherwise no response or avoidance to APM (−) against tap water. The smaller sized animals were offered the choice of two bottles attached to the cage. The medium-sized animals were provided with two larger drinking bowls, which were placed inside the cages. The Hylobatidae and Pongidae were tested with the aid of their usual drinking mugs. So, all animals were able to choose between an APM solution and tap water. We randomly changed the side of the offered APM solution. Starting the tests early in the morning, the animals were deprived of fluid intake since the evening before. Therefore, each animal was in a thirsty condition. Further information you will find by Glaser (1968, 1979), and by Glaser and Hellekant (1977).

The intake of 10 APM solutions ranking from 0.1 to 30.0 mM and water was measured in *Tupaia belangeri*, closely related to the prosimians, and fourteen primate (sub-) species (*Eulemur mongoz, Loris tardigradus, Callithrix jacchus jacchus, Callithrix jacchus penicillata, Callithrix j. j.* × *Callithrix j.p., Cebuella pygmaea pygmaea, Cebuella pygmaea niveiventris, Saguinus midas niger, Saguinus oedipus oedipus, Callimico goeldii, Aotus trivirgatus, Macaca mulatta, Cercocebus atys atys, Cercopithecus nictitans stampflii*). These experiments were carried out in the primate facility of the Anthropological Institute Zürich. In Hylobatidae and Pongidae the intake of 0.45, 1.8 and 7.2 mM was controlled and further, the responsiveness to 3.3 mM APM in totally 70 (sub-)

species of primates, out of approximately 180 which are living today. These studies were conducted in the Zoological Gardens of Frankfurt (Germany), Mulhouse (France), Zürich (Switzerland) and on the Affenberg Salem (Germany), in the Zoological Station Eichberg (Switzerland), and further experiments were conducted in the primate facilities of Ciba-Geigy and Hoffmann-LaRoche in Basel (Switzerland).

Earlier observations (Steiner and Glaser, 1984; Glaser *et al.*, 1987) and video recording analysis (Glaser and Steiner, 1983, 1985; Glaser *et al.*, 1985) were a help in judging primates' behaviour (facial expressions like sampling-sipping, lapping or eager drinking, quick swallow, mouth open, lips apart, sucking-smacking, head orientating towards stimulus) when tasting a sweet solution that was clearly differentiable from those patterns of behaviour triggered by other qualities or simply tap water (e.g. mouth corners down, spitting, head turn/head shake, gaping, head withdrawal from stimulus). These patterns have shown nearly all primates tested and it is not a species specific behaviour.

Results

When we ascertained the sweet taste detection thresholds in three species of the subfamily Cercopithecidae (Table II), which all preferred APM against tap water, it was found for example in *Macaca mulatta* ($n = 6$) at the threshold value of 0.5 mM that on the average 83.7% (SD ± 13.2) of the total consumption of fluid in 20 days was APM. The highest preferred test solution in *Macaca mulatta* was a solution of 10.0 mM and here the average of 97.1% (SD ± 7.1) of the total consumption of fluid within 10 days was APM.

Furthermore, when we tested 10 different levels of APM in the range of 0.1−30.0 mM we found that neither *Tupaia belangeri* nor *Eulemur mongoz, Loris tardigradus, Callithrix jacchus jacchus, Callithrix jacchus penicillata, Callithrix j.j. × Callithrix j. p., Cebuella pygmaea pygmaea, Cebuella pygmaea niveiventris, Saguinus midas niger, Saguinus oedipus oedipus, Callimico goeldii* and *Aotus trivirgatus* show a preference to all these applicated APM-concentrations. These findings may be demonstrated for example in *Eulemur mongoz*. The 'most accepted' test solution in this species was a concentration of 5.0 mM and this means that only an average of 51.3% (SD ± 23.7) of the total consumption of fluid was APM.

Finally, all results obtained in collaboration with Zoological gardens and other primate facilities indicate that APM, which elicits a strong sweet sensation in man, is not preferred to tap water by either the common tree shrews (*Tupaia belangeri*), or fourteen species of the Prosimii (total: 53 animals), including the Philippine tarsier (*Tarsius*

Table II. Taste thresholds (in 10^{-3} mM) for aspartame

Species		Authors
Homo sapiens sapiens	0.027	Mazur and Ripper (1979)
Macaca fuscata	0.3	Sato *et al.* (1977)
Macaca mulatta ($n = 6$)	0.5	Glaser
Cercocebus atys atys ($n = 1$)	0.6	Glaser
Cercopithecus nictitans stampflii ($n = 1$)	0.5	Glaser

Table III. Responses of Tupaiidae and Prosimii to 3.3 mM aspartame. (e = electrophysiological; b = TBP data; *n* = number of animals tested; *r* = reactions: − = no response or avoidance, + = preference). The *e*-value in *Macaca fuscata* is from Sato *et al.*, 1977, all other *e*-data from Hellekant *et al.*, 1976, 1981, 1985, 1986, 1990 and Brouwer *et al.*, 1983). All TBP data are from Glaser

	Aspartame-reactions			
	Species	e/b	*n*	*r*
Prosimii	*Tarsius syrichta carbonarius*	b	2	−
	Galago senegalensis	e/b	1	−
	Nycticebus coucang	e/b	1	−
	Loris tardigradus	b	2	−
	Microcebus murinus	e/b	8	−
	Eulemur rubriventer	b	2	−
	Eulemur coronatus	b	2	−
	Eulemur mongoz	e/b	5	−
	Eulemur fulvus albifrons	b	7	−
	Eulemur macaco flavifrons	b	2	−
	Eulemur macaca macaco	b	6	−
	Varecia variegata rubra	b	5	−
	Varecia variegata variegata	b	3	−
	Lemur catta	b	7	−
Tupaiidae	*Tupaia belangeri*	e/b	5	−

syrichta carbonarius) see Table III, or the group of 23 (sub-) species of South American Platyrrhini (total: 112 animals) see Table IV.

From a phylogenetic point of view the first group within the order of primates, which respond to aspartame in the same way as man, is the superfamily Cercopithecoidea, the Old World monkeys, tested in 22 (sub-) species (total: 106 animals) see Table V. Also the lesser apes, the Hylobatidae with four species (total: 13 animals) and the great apes, the Pongidae with five (sub-) species (total: 25 animals) preferred APM to water. This means that all the catarrhine primates prefer APM to tap water (see Table VI).

Discussion

All these observations demonstrate that in aspartame we have a second compound which from a phylogenetic point of view shows characteristics similar to thaumatin, which constitutes a clear dichotomy within the order Primates at the same intersecting line (Glaser *et al.*, 1978; Figure 1).

Turning to taste hypothesis to explain the origin of New World monkeys, an answer to this problem is not in sight. The physiological feature of tasting thaumatin and APM does not exist in any South American primate. Therefore, the Platyrrhini had split off before the capability to respond to thaumatin and APM evolved. This means, taking into account the finding of *Oligopithecus* in Fayum, that the ability to discriminate thaumatin and APM must have been present as early as about 33 million years ago. If we assume that *Oligopithecus* had already undergone a certain development, this thaumatin/AMP discriminating feature may be about 38 million years old. This is in good agreement with time scales postulated. However, the recent findings disagree with

Table IV. Responses of the South American Platyrrhini to 3.3 mM aspartame (see explanation Table III)

	Species	e/b	*n*	*r*
Cebidae	*Ateles geoffroyi geoffroyi*	b	2	—
	Ateles fuscipes robustus	b	2	—
	Saimiri sciureus	b	11	—
	Cebus apella xanthosternos	b	3	—
	Cebus capucinus capucinus	b	1	—
	Chiropotes satanas chiropotes	b	2	—
	Pithecia pithecia	b	3	—
	Aotus trivirgatus	b	4	—
Callimiconinae	*Callimico goeldii*	b	11	—
Callitrichidae	*Leontopithecus rosalia rosalia*	b	5	—
	Saguinus oedipus oedipus	b	2	—
	Saguinus i. i. × *Saguinus i. s.*	b	4	—
	Saguinus i. subgrisescens	b	2	—
	Saguinus imperator imperator	b	3	—
	Saguinus labiatus labiatus	b	10	—
	Saguinus midas niger	e/b	6	—
	Saguinus midas midas	b	7	—
	Cebuella p. niveiventris	b	1	—
	Cebuella pygmaea pygmaea	b	8	—
	Callithrix jacchus geoffroyi	b	2	—
	Callithrix j. j. × *Callithrix j. p.*	b	1	—
	Callithrix j. penicillata	b	2	—
	Callithrix jacchus jacchus	b	20	—

the hypothesis that movement of animals during the late Eocene, Oligocene and Miocene would have been a process of island-hopping which could have been achieved by swimming, fording or rafting. The connection between North and South America seems more reasonable (Glaser, 1989).

Another question arises—what kind of mechanism is responsible for this phylogenetic separation?

The similar effect with thaumatin and APM occurs, although we have two very different molecules. On the one hand the large molecules of thaumatin, the sweet-tasting protein from the West African fruit *Thaumatococcus daniellii* (Marantaceae) with a sweetness potency in man of about 100 000 times that of sucrose, on the molar basis. The structure of thaumatin (Figure 2) consists of a single polypeptide chain of 207 amino acids (18 different amino acids) with a molecular mass of approximately 22 000 dalton, and this is a natural compound (van der Wel and Ledeboer, 1989). On the other hand APM, a synthetic compound, is a small molecule with a molecular weight of 294 dalton (Mazur *et al.*, 1969).

However, maybe these two totally different molecules must have a same sweet unit (site) fitting to the corresponding receptor site, but that sweet unit may be different, e.g. for sucrose.

If you compare the crystal structures of thaumatin (Figure 2) and monellin (Figure 3),

Table V. Responses of the Old World monkeys, the Cercopithecoidea, to 3.3 mM aspartame (see explanation Table III)

	Species	e/b	*n*	*r*
Colobinae	*Presbytis obscura*	b	3	+
Cercopithecinae	*Erythrocebus patas patas*	b	3	+
	Cercopithecus nigroviridis	b	2	+
	Cercopithecus talapoin	b	5	+
	Cercopithecus erythrogaster	b	2	+
	Cercopithecus nictitans stampflii	b	1	+
	Cercopithecus hamlyni	b	3	+
	Cercopithecus preussi	b	1	+
	Cercopithecus l'hoesti	b	5	+
	Cercopithecus diana roloway	b	3	+
	Cercopithecus diana diana	b	1	+
	Cercopithecus aethiops	b	5	+
	Theropithecus gelada	b	8	+
	Papio papio	b	2	+
	Papio anubis	e/b	6	+
	Cercocebus atys atys	b	1	+
	Macaca tonkeana	b	4	+
	Macaca nigra	b	7	+
	Macaca fuscata	e/b	20	+
	Macaca arctoides	b	6	+
	Macaca mulatta	e/b	10	+
	Macaca sylvanus	b	8	+

Table VI. Responses of the Hominoidea to 3.3 mM aspartame (see explanation Table III)

	Species	e/b	*n*	*r*
Hominidae	*Homo sapiens sapiens*	b	∞	+
Pongidae	*Gorilla gorilla gorilla*	b	7	+
	Pan paniscus	b	2	+
	Pan troglodytes troglodytes	e/b	10	+
	Pongo pygmaeus abelii	b	4	+
	Pongo pygmaeus pygmaeus	b	2	+
Hylobatidae	*Symphalangus syndactylus*	b	3	+
	Hylobates lar lar	e	4	+
	Hylobates pileatus	b	4	+
	Hylobates concolor	b	2	+

another sweet-tasting protein from the African fruit *Dioscoreophyllum cumminsii* (Menispermaceae), with nearly the same sweetness potency to man as thaumatin, you find five pairs of homologous tripeptide sequences (Kim *et al.*, 1988). The five tripeptides in thaumatin and monellin labelled 1 to 5 in the figures. However, thaumatin and monellin have only two small regions (1 and 4; both tripeptides), located in exposed, looping regions, sharing the same sequence and topology. Maybe the possibility is that

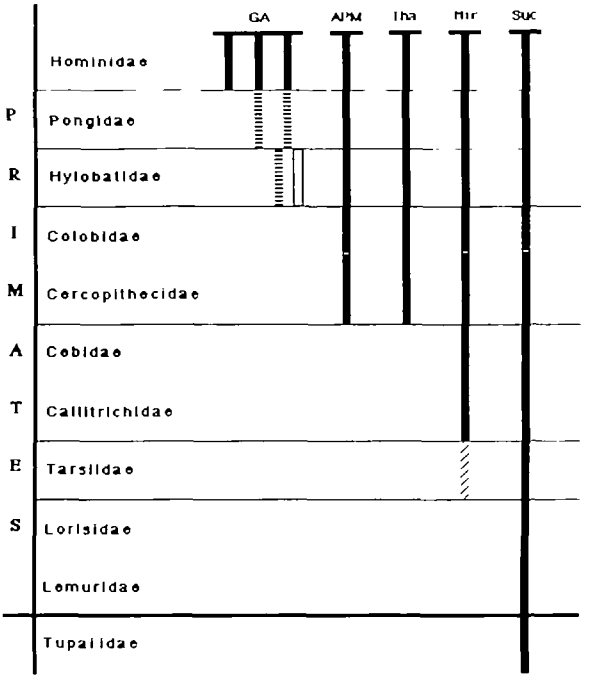

Fig. 1. Scheme of responses in primates of sucrose (Suc), miraculin (Mir), thaumatin (Tha), aspartame (APM) and gymnemic acid (GA). (Black bar = same responses as man; hatched bar = percentage lower responses versus man; white bar or white = no response or avoidance to the stimuli mentioned; after Hellekant, 1977.)

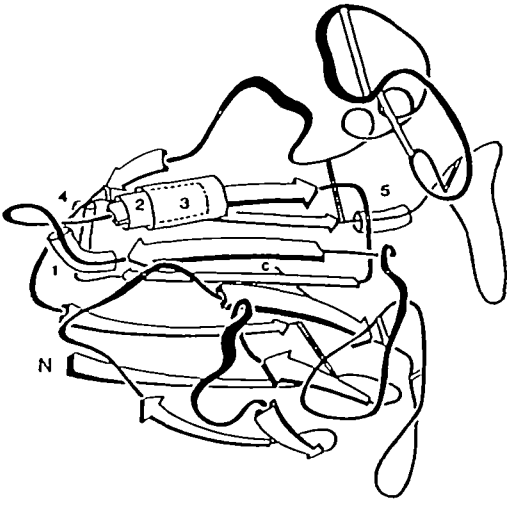

Fig. 2. Schematic drawing of the backbone structure of thaumatin (from Kim *et al.*, 1988).

the active unit of these small exposed regions are nearly similar—or react nearly similarly—like the active sweet unit of aspartame (Figure 4) (van der Heijden *et al.*, 1979).

Nevertheless, our results within the order Primates show that there must exist more than one different receptor type sensitive to sweet, because we have found one primate group which is sensitive to sucrose and to thaumatin and APM, and another group which is only sensitive to sucrose and not to thaumatin and APM.

The conclusion may be that the receptor sites accepting sweet tasting molecules to man are different across the primates tested here and are unique inside each group. However, there still remains the question: is there a small family of sweet taste receptor proteins or is there one protein with recognition sites for so many structurally diverse sweet components?

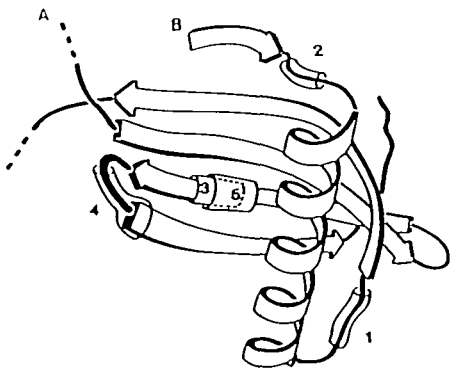

Fig. 3. The backbone structure of monellin (from Kim *et al.*, 1988). The numbered 'sleeves' are the regions of sequence homology with thaumatin.

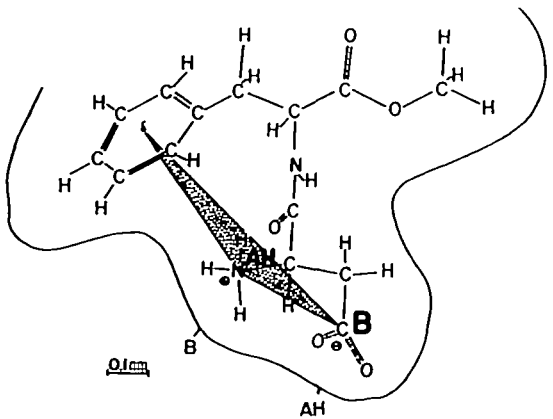

Fig. 4. Proposed conformation of aspartame during interaction with the receptor site (from van der Heijden *et al.*, 1979).

A new model giving the molecular features which allow a molecule to initiate a sweet sensation in man is described recently by Tinti and Nofre (1991). This model assumes the existence on the sweet receptor (a protein) of at least eight recognition sites with the capacity to identify in sweeteners at least eight optional interaction sites. Maybe during the evolution the number of allosteric recognition sites have been extended and only man and all catarrhine primates have eight recognition sites on the receptor protein.

If you have in mind results with PTC (Phenylthiocarbamide), you will find in one human population taster and non-taster for bitter (Glaser, 1972). Maybe this is a suggestion that we have in the quality bitter within one species different receptor types too.

However, at least more knowledge is necessary concerning what happened on the surface of the active receptor site, to explain all reactions involved in taste sensitivity.

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